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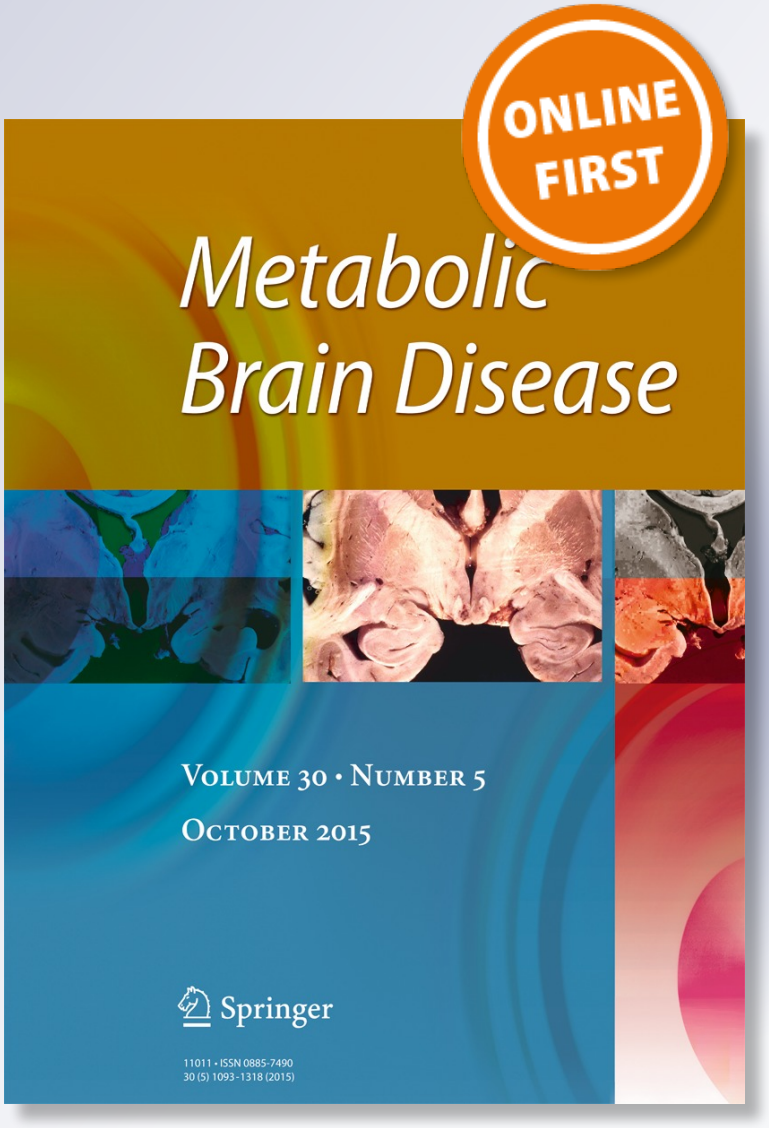

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ORIGINAL ARTICLE

Clinical science workshop: targeting the gut-liver-brain axis

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Abstract A clinical science workshop was held at the ISHEN meeting in London on Friday 11th September 2014 with the aim of thrashing out how we might translate what we know about the central role of the gut-liver-brain axis into targets which we can use in the treatment of hepatic encephalopathy (HE). This review summarises the integral role that inter-organ ammonia metabolism plays in the pathogenesis of HE with specific discussion of the roles that the small and large intestine, liver, brain, kidney and muscle assume in ammonia and glutamine metabolism. Most recently, the salivary and gut microbiome have been shown to underpin the pathophysiological changes which culminate in HE and patients with advanced cirrhosis present with enteric dysbiosis with small bowel bacterial overgrowth and translocation of bacteria and their products across a leaky gut epithelial barrier. Resident macrophages within the liver are able to sense bacterial degradation products initiating a pro-inflammatory response within the hepatic parenchyma and release of cytokines such as tumour necrosis factor alpha (TNF-α) and interleukin-8 into the systemic circulation. The endotoxemia and systemic inflammatory response that are generated predispose both to the development of infection as well as the manifestation of covert and overt HE. Co-morbidities such as diabetes and insulin resistance, which commonly accompany cirrhosis, may promote slow gut transit, promote bacterial overgrowth and increase glutaminase activity and may need to be acknowledged in HE risk stratification assessments and therapeutic regimens. Therapies are discussed which target ammonia production, utilisation or excretion at an individual organ level, or which reduce systemic inflammation and endotoxemia which are known to exacerbate the cerebral effects of ammonia in HE. The ideal therapeutic strategy would be to use an agent that can reduce hyperammonemia and reduce systemic inflammation or perhaps to adopt a combination of therapies that can address both.

Keywords Hepatic Encephalopathy · Ammonia · Gut · Liver · Brain · Muscle · Inflammation

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Inter-organ ammonia and glutamine metabolism in hepatic encephalopathy

Ammonia has for decades been considered to be central in the pathogenesis of HE. Ammonia is neurotoxic and its accumulation in the context of liver disease is multi-factorial and involves multiple-organ systems. The liver is the predominant ammonia-detoxifying organ in the human body. The majority of circulating ammonia arises from intestinal breakdown of ingested amino acids and urea. In cirrhotic patients, this process is augmented due to increased enterocyte expression of enterocyte phosphate-activated glutaminase (PAG) (Romero-Gomez et al. 2004). Ammonia-rich blood reaches the liver via the portal circulation and detoxification occurs in the liver through either the incorporation of ammonia in the synthesis of urea in peri-portal hepatocytes or alternatively, conversion to glutamine by glutamine synthetase (GS) expressing perivenous hepatocytes (Haussinger et al. 1992). In a healthy liver, these mechanisms remove almost all ammonia. The absence of hepatic GS was in a recent knock-out mouse model

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demonstrated sufficient to cause systemic hyperammonemia (Qvartskhava et al. 2015). Indeed, in a cirrhotic liver where both urea and glutamine synthesis are diminished by a reduced synthetic capacity and porto-systemic shunts allow ammonia to bypass the liver, ammonia inevitably evades hepatic detoxification and accumulates (Fig. 1).

In acute liver failure (ALF), rapid hepatic parenchymal destruction causes a steep increase in ammonia as counter-regulatory mechanisms have little time to take effect. Increased arterial ammonia is reflected in increased intracerebral ammonia (Sorensen and Keiding 2007). Ammonia is thought to cross the blood brain barrier (BBB) by means of both passive diffusion and active transport (Nagaraja and Brookes 1998). Within the brain, hyperammonemia disturbs neurotransmission. High levels of ammonia cause excessive N-methyl D-aspartate (NMDA) receptor activation, which has been linked to adenosine triphosphate (ATP) depletion and neuronal death (Bosoi and Rose 2009; Hermenegildo et al. 2000). In chronic hyperammonemia, adaptations lead to decreased cyclic guanosine monophosphate (cGMP) production following NMDA receptor ligation, which is protective but simultaneously slows learning abilities (Erceg et al. 2006; Hermenegildo et al. 1998). Also a high gamma-aminobutyric acid (GABA) tone has been demonstrated in the cerebellum of hyperammonemic rats with neuroinhibitory consequences (Cauli et al. 2009). Although glutamine is a central precursor of this major inhibitory neurotransmitter, the mechanism perceived to lead to an increase in GABAergic tone per se probably relates to the ammonia-induced production of neurosteroids by astrocytes, which activate GABA receptors (Ahboucha et al. 2006; Jones 2003). Both GABA and NMDA receptor blockers have been shown to be protective in animal models of HE (Cauli et al. 2009).

Astrocytes take part in the BBB and serve to protect the neurons from the toxic effects of ammonia. Like the perivenous hepatocytes, the astrocytes express GS and can thereby convert ammonia and glutamate into glutamine (Martinez-Hernandez et al. 1977). The accumulation of glutamine, albeit not directly toxic, may adversely influence cerebral function. Firstly, glutamine functions as an osmolyte inducing astrocyte swelling as astrocytes attempts to osmoregulate (Blei et al. 1994; Haussinger et al. 2000). This phenomenon is most pronounced in ALF due to rapid ammonia accumulation, but in chronic liver disease increased brain water is also detectable on MRI imaging (Cordoba et al. 2001). Secondly, glutamine in excess is transported into mitochondria and broken down to glutamate and ammonia again; the latter causing oxidative stress and induction of mitochondrial permeability transition (MPT). This may create a vicious cycle of free radical formation causing further mitochondrial damage (Bai et al. 2001).

Ammonia metabolism also occurs in skeletal muscles, which richly express GS. In patients managed with transjugular intrahepatic portosystemic shunts (TIPSS), the muscle is the predominant ammonia conversion site. However, the amount of nitrogen incorporated into glutamine exceeds that incorporated into ammonia, and any ammonia detoxification is only temporary as kidneys and the gut rich in glutaminase break down glutamine again (Olde Damink et al. 2002). The only net removal of nitrogen by muscle is performed by alanine aminotransferase, which converts pyruvate to alanine which is then metabolized to urea in the liver (Felig et al. 1969). The kidneys contain both glutaminase and GS, but are normally a net producer of ammonia with 70 % of

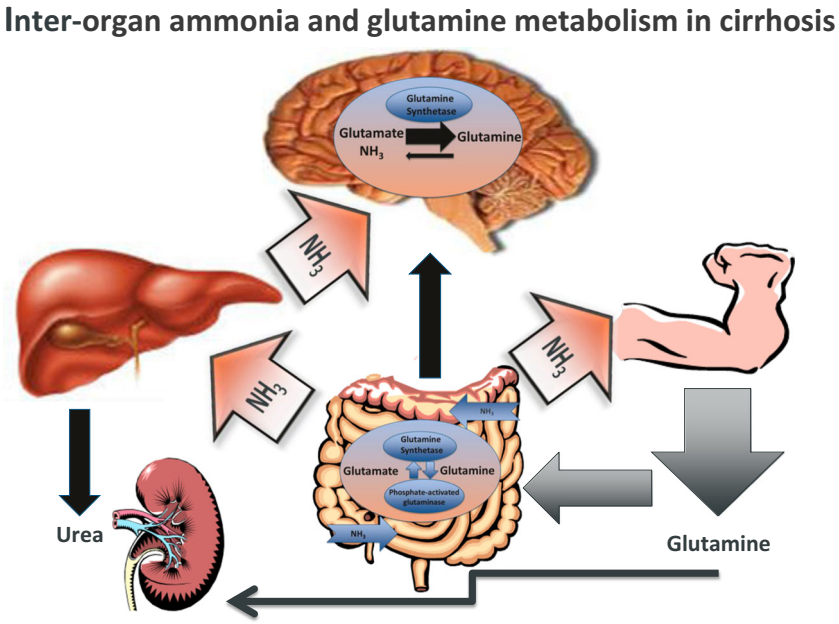

Fig. 1 Inter-organ ammonia and glutamine metabolism in cirrhosis

this being reabsorbed back into circulation and only 30 % excreted in the urine (Olde Damink et al. 2002). When an upper gastrointestinal bleed precipitates hyperammonemia in patients with cirrhosis, renal production of ammonia is the predominant source (Olde Damink et al. 2003). In some patients with cirrhosis, the kidneys can become net ammonia excretors during hyperammonemia which can be utilized as a therapeutic target following volume expansion with normal saline solution in stable patients with cirrhosis (Jalan and Kapoor 2003).

Systemic inflammation and hepatic encephalopathy

Local hepatic inflammation is a feature of both ALF and cirrhosis. The liver coordinates and participates in vital immunological functions given its anatomical location and vascular supply resulting in exposure to an immense number of antigens from the gut. In ALF, sudden and marked hepatic destruction and necrosis causes profound hepatic inflammation (Streetz et al. 2000). In cirrhosis, bacterial overgrowth in the gut increases the antigen load, which in combination with ongoing liver damage is thought to trigger immune activation in the liver. Systemic inflammation may also arise from gut-derived antigens bypassing the liver due to portosystemic shunting (Cirera et al. 2001). Exhaustion of immune effector cells is a plausible result of this overwhelming effect. Indeed, neutrophils demonstrate impaired bactericidal capacity in both ALF and cirrhosis and this predicts the development of organ dysfunction, infection and mortality (Taylor et al. 2013, 2014). Monocytes have also been shown to exhibit reduced antigen presenting capabilities and TNF-α production in liver failure (Berry et al. 2011). This renders these patients susceptible to infection, which may further exacerbate the pro-inflammatory state (Caly and Strauss 1993).

In ALF, there is a robust correlation between levels of arterial ammonia and the severity of HE with levels greater than 150 μmol/L causing the most adverse phenotype of HE with the rapid onset of cerebral edema, intracranial hypertension and the progression to cerebral herniation in up to 25 % of patients (Bernal et al. 2007; Clemmesen et al. 1999). Yet in chronic liver disease, the severity and manifestation of HE is far more variable and sensitive to the presence of precipitating factors (Vilstrup et al. 2014). Indeed, in cirrhosis although HE grade somewhat parallels increasing arterial ammonia concentration, hyperammonemia can be detected in patients without HE and likewise normal ammonia levels can be found in those exhibiting grade III/IV HE (Ong et al. 2003; Shawcross et al. 2011). An increasing body of evidence points to infection, inflammation and systemic oxidative stress being important in modulating or even precipitating HE (Rolando et al. 2000; Shawcross et al. 2004; Takada et al. 2001). In ALF, development of infection and systemic inflammation is associated with the progression of HE to more advanced stages (Rolando et al. 2000; Vaquero et al. 2003).

In cirrhosis, a strong correlation between levels of TNF-α and HE exist with TNF-α concentration independently predicting the severity of HE (Odeh et al. 2005). Furthermore, resolution of inflammation improves cognitive and motor function in HE. Induced hyperammonemia in infected patients with cirrhosis worsened neuropsychological function whilst there was evidence of a systemic inflammatory response syndrome (SIRS) but not following resolution of infection despite hyperammonemia (Shawcross et al. 2004). Furthermore, in portocaval shunt operated rats non-steroidal anti-inflammatory treatment with ibuprofen improved cognitive impairment. This together supports a synergistic effect of ammonia and inflammation in HE (Cauli et al. 2007; Wright et al. 2007a).

Circulating cytokines such as TNF- α and interleukin-1β induce nitric oxide (NO) and prostanoid synthesis in endothelial cells eliciting secretion of pro-inflammatory cytokines by microglia and astrocytes (Romero et al. 1996). Cytokines can also be directly exported across the BBB (Banks et al. 1991, 1994). Brain autopsies from patients with cirrhosis who died of HE also demonstrate microglial activation (Zemtsova et al. 2011) and brain cytokine flux measured in patients with ALF support an intrathecal cytokine production (Wright et al. 2007b). Moreover, inflammatory hyperemia could increase ammonia delivery to the brain (Jalan et al. 2004).

Exaggerated production of reactive oxygen species (ROS) by neutrophils may also add to the state of systemic oxidative stress, which arises from the unbalanced release of oxidant and antioxidant proteins by the diseased liver (Chen et al. 1997). In patients with cirrhosis with similar ammonia levels, those with minimal HE have higher plasma levels of systemic oxidative stress markers than patients without (Montoliu et al. 2011). Oxidative stress is shown to increase brain water in concert with ammonia and may thereby be involved in the pathogenesis of HE (Bosoi et al. 2012).

Etiologically and mechanistically, ammonia and inflammation are clearly intertwined concepts in the setting of HE. Systemically, ammonia can in itself act upon neutrophils to increase their ROS production and lower their ability to phagocytose bacteria (Shawcross et al. 2008). In terms of brain inflammation, ammonia can directly or via oxidative stress induced by MPT or lactate accumulation activate microglia (Jiang et al. 2009). Furthermore, induction of heme-oxygenase-1 (HO-1) and inducible nitric oxide synthase (iNOS) can be demonstrated in astrocyte cultures exposed to a combination of ammonia and pro-inflammatory cytokines (Chastre et al. 2010). Thus, both ammonia and inflammation must be kept in mind when searching for efficacious treatment strategies in HE.

Reduced production of antioxidants as the liver's synthesis capacity decreases combined with an increased production of ROS from hepatocyte destruction, neutrophil dysfunction etc.

creates a state of systemic oxidative stress which like systemic inflammation may be an important modulating factor in HE development (Bosoi et al. 2012).

The role of the gut as the driver of systemic inflammation in hepatic encephalopathy

Human beings are colonized by a massive variety of microorganisms collectively known as the human microbiota, which consist of bacteria, fungi, viruses, archaea (single-celled prokaryotes) and protozoa (Morgan et al. 2013). The composite of the genes that are harbored by these microorganisms and the environmental milieu within which they reside and interact is referred to as the human microbiome (Turnbaugh et al. 2007). The gastrointestinal tract is of most relevance to liver pathology not only because of its intimate anatomical relationship to the liver via the portal vein, but the quantum difference in diversity and sheer number of microbial species contained within the gut compared to other sites. The gut microbiota consists of tens of trillions of microorganisms which outnumber human cells by a factor of ten and weigh in at up to 2 kg en masse (Baquero and Nombela 2012). About two-thirds of the entire gut microbiome is unique to each individual, and the genomic content of these microbes as a whole consists of over three million genes, a staggering 150 times the amount contained within the entire human genome (Human Microbiome Project 2012; Qin et al. 2010). The gut microbiota consists of over 1,000 species of bacteria, but only up to 170 species predominate in any given individual with *Bacteroidetes* and *Firmicutes* being the dominant phyla (Lozupone et al. 2012).

The density of microbiota increases significantly in the jejunum and ileum in comparison to the gastric cavity and duodenum. However, it is within the colon that the most densely populated area is to be found, where there are over 1000 colony forming units/mL mainly composed of anaerobes such *Bacteroides*, *Porphyromonas*, *Bifidobacterium*, *Lactobacillus*, and *Clostridium*. These anaerobic bacteria outnumber aerobic bacteria by a factor of 100 to 1000:1 due to the low concentrations of oxygen within the colon, and have evolved to thrive in this hostile ecosystem. The composition of the gut microbiota varies within the intestinal lumen depending on the level of the gut being interrogated and the luminal diameter at that point, with certain microorganisms more adherent to the mucosal surface whilst others predominate in the lumen itself and are more representative of the composition measured from fecal samples.

Whilst the complex and synergistic role of gut flora and its relationship with the human host is still being studied, advanced by the recent explosion in high throughput culture-independent sequencing and genomic techniques, it is now known that the gut microbiota have essential functions in complex metabolic pathways, nutrition, homeostasis and the development and maintenance of the innate and adaptive immune systems (Guarner and Malagelada 2003). In particular, the production of short-chain fatty acids (SCFAs) from dietary starches which are otherwise indigestible, mainly by bacteria from the *Bacteroidetes* phylum, have anti-inflammatory and immune signaling properties as well as acting as a vital substrate for energy production for intestinal mucosal cells which helps to maintain barrier function and intestinal integrity (Smith et al. 2013). Disturbances in these mechanisms have direct implications in the development and propagation of a multitude of liver-centric disease processes (Cenit et al. 2014; Owyang and Wu 2014; Shreiner et al. 2015).

It is well established that those afflicted with cirrhosis have a multitude of intestinal factors that lead to downstream deleterious local and systemic effects and play a major role in clinically adverse outcomes including HE. This is of course in part due to the delivery of gut-derived bacteria and their products directly to the liver via the portal vein, with the liver being of paramount importance in needing to generate an effective innate immune response.

Small bowel bacterial overgrowth in combination with translocation of these bacteria and their endotoxins (such as lipopolysaccharide, flagellin, peptidoglycan and bacterial DNA) can cross a more permeable gut epithelial membrane increasing exposure of the liver to immune-activating bacterial degradation products. This is further exacerbated by underlying portal hypertension and endothelial dysfunction (Wiest and Garcia-Tsao 2005), whilst portosystemic shunting increases the delivery of these bacterial products to the systemic circulation and so evading the reticuloendothelial system (Cirera et al. 2001).

Endotoxins activate hepatic macrophages via toll-like receptor (TLR) signaling, stimulating the production of pro-inflammatory cytokines such as TNF-α and interleukin-8 which in turn trigger the hepatic migration of neutrophils and monocytes (Singh et al. 2011). This ultimately culminates in hepatic injury and systemic inflammation, and further induces innate immune dysfunction predisposing to infection and development of decompensating complications such as hemorrhage, sepsis and HE (Bajaj et al. 2014b).

Whilst small bowel bacterial overgrowth, increased intestinal permeability, translocation of bacteria and their products and systemic inflammation are all intimately linked and contribute to HE (Fig. 2), more recently the concept and confirmation of enteric dysbiosis in cirrhosis has attracted interest and scrutiny. Adverse changes in gut microbiota have been causally linked to the pathogenesis of cirrhosis and the progression to advanced liver disease. (Nolan 2010) Quantitative metagenomic analyses have recently been employed to demonstrate that 75,245 microbial genes differ in abundance between patients with cirrhosis and healthy individuals (Qin et al. 2014).

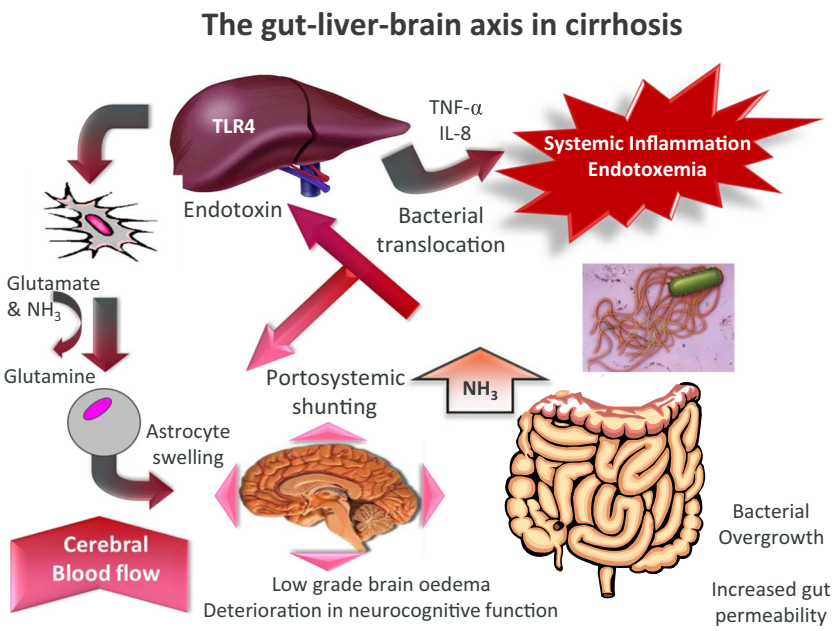

Fig. 2 The gut-liver-brain axis in cirrhosis

Changes in bacterial composition characterised by a relative decrease in potentially beneficial autochthonous taxa as well as a relative overgrowth of potentially pathogenic taxa (such as *Staphylococcaeae, Enterobacteriaceae*, and *Enterococcaceae*) was independently associated with severity of liver disease and the development of endotoxemia and HE, suggesting a direct pathogenic role (Bajaj 2014; Bajaj et al. 2012a, 2014b; Chen et al. 2011; Nava and Stappenbeck 2011). The pathophysiological mechanisms underpinning this are complex, and it has been suggested that the reduced production of SCFAs, anti-bacterial peptides and changes in bile acid production resulting from enteric dysbiosis all contribute to worsening disease severity. Indeed, it is widely recognised that intestinal decontamination with non-absorbable antibiotics such as rifaximin is an effective treatment for covert and overt HE (Bass et al. 2010; Sidhu et al. 2011), directly implicating gut dysbiosis in the development of neurocognitive dysfunction.

Salivary dysbiosis is also present and is likely to an extent to be a reflection of those pathological changes also seen affecting the more distal intestinal microbiota in cirrhosis, with an associated systemic and salivary-specific inflammatory milieu (Bajaj et al. 2015). Salivary dysbiosis was more pronounced in over a third of cirrhotic patients that went on to require liver-related hospital admissions during the 90-day follow-up period, suggesting a causative role particularly when considering that saliva from patients with cirrhosis is enriched with genes related to endotoxin synthesis proteins, and nucleic acid and vitamin metabolism, the latter of which are purported to modulate intestinal barrier integrity and oxidative stress. This in combination with evidence of a buccal pro-inflammatory milieu with higher salivary interleukin-1β and interleukin-6 (IL-6) concentrations and a resultant increase in secretory IgA, accompanied by a reduction in histatins 1 and 5 and lysozyme, are in keeping with impairment of local innate defenses of the oral cavity. It must be said however that the contribution of salivary dysbiosis compared to the distal gut in cirrhosis is likely to be minimal, mainly because of the quantum difference in the number of bacteria between the two sites. The gut and related enteric dysbiosis is thus the main driver of inflammation, with this organ being the main target when considering therapeutic options such as non-absorbable antibiotics like rifaximin.

One drawback of studies analyzing the gut microbiome in patients with cirrhosis is that geographical differences in its composition and function may be vastly disparate and therefore the location of the study e.g. China versus the USA may make characterization of gut dysbiosis country and environment-specific.

Therapeutic strategies targeting the gut-liver-brain axis

Traditionally therapies have looked to reduce ammonia by utilising the gut as a target organ, starting with neomycin in the 1970's and quickly followed by the non-absorbable disaccharides such as lactulose and lacitol. These remain the mainstay of treatment in primary and secondary care (Blei and Cordoba 2001). Non-absorbable disaccharides reduce circulating ammonia by two main mechanisms; the acidification of the gut lumen prevents urease-producing gut bacteria from thriving thereby reducing ammonia production. This, coupled with inhibition of ammonia diffusion from the lumen into the circulating blood stream, effectively reduces the overall ammonia level. Table 1 summarises these and the other therapeutic strategies that have been used to manage HE in targeting

Table 1 Therapeutic strategies targeting the gut-liver-brain axis

Treatment	Target	Effect	Role in ALF	Role in OHE	Role in MHE
Non-absorbable Disaccharides *	Enteric bacteria	Reduced ammonia production and reduction of diffusion into circulation	No	Yes	Yes
Polyethylene glycol*	Enteric bacteria	Reduced ammonia production and reduction of diffusion into circulation	No	Yes	No
Rifaximin*	Enteric bacteria	Modulation of microbiota and anti-inflammatory	No	Yes	Yes
Probiotics*	Enteric Bacteria	Modulation of microbiota and anti-inflammatory Reduced ammonia production	No	?	Yes
Branched chain amino acids*	Liver/Muscle	Increased ammonia metabolism	No	Yes	Yes
L-Ornithine L-Aspartate*	Liver/Muscle	Increased ammonia metabolism	No	Yes	Yes
Ornithine Phenylacetate	Liver/Muscle	Increased ammonia metabolism and excretion	Yes	Yes	?
Glyceryl Phenylbutyrate	Liver	Increased ammonia metabolism and excretion	No	Yes	?
Metformin*	Liver/Muscle	Decreases glutaminase activity	No	Yes	Yes
Albumin dialysis*	Humoral factors	Anti-inflammatory and anti-oxidant	Yes	Yes	No
Plasmapheresis*	Humoral factors	Anti-inflammatory and anti-oxidant	Yes	Yes	No

Therapies in routine clinical use are asterisked *

elements of the gut-liver-brain axis. Of note, only a handful of these therapies are available and recognised for routine use in the clinical setting.

Although the utility of lactulose as first line in the treatment of acute HE has been hotly debated (Als-Nielsen et al. 2004; Shawcross and Jalan 2004) in the absence of any large multicentre randomized controlled trial ever being performed, there are robust data to support its use in the secondary prophylaxis of recurrence of overt HE (Sharma et al. 2009) and in the treatment of covert HE (Prasad et al. 2007). Recently, the hepatic encephalopathy: lactulose vs polyethylene glycol (HELP) trial recently showed statistically significant reduction in both HE grade and resolution of symptoms when polyethylene glycol (PEG) solution was used instead of lactulose (Rahimi et al. 2014). The increase in renal ammonia excretion as a consequence of administering 4 l of PEG solution could however explain its non-inferiority compared to lactulose in this trial.

A Cochrane systemic review and meta-analysis favoured antibiotics such as vancomycin, neomycin and metronidazole over non-absorbable disaccharides however their oto-, nephro- and neurotoxicities have precluded long term use (Als-Nielsen et al. 2004). Rifaximin, is a broad-spectrum antibiotic which has minimal systemic absorption and has recently taken the place of the other non-absorbable antibiotics after a large double-blinded randomised controlled trial of 299 patients demonstrated an improvement in maintained remission from HE and a reduction in hospitalisations due to HE over a 6 month period in patients with cirrhosis who were administered rifaximin versus placebo (Bass et al. 2010). Rifaximin has also been examined in the context of patients with covert HE and significantly improved driving simulator performances compared to the placebo group (Bajaj et al. 2011). Ammonia levels have never convincingly been shown to drop following rifaximin therapy and increased levels of the anti-inflammatory cytokine interleukin-10 have been identified in the rifaximin-treated patients which may allude to its mechanism of action being an anti-inflammatory rather than ammonia-lowering in nature. Furthermore, rifaximin has been demonstrated to reduce systemic endotoxin levels following 8 weeks of therapy changing the function but not the composition of the gut microbiome (Bajaj et al. 2013). Interestingly, a UK multicentre retrospective study including 170 patients published in abstract form and presented at the workshop has reported that rifaximin-α therapy given for 3 months significantly reduced hospital re-admission rates, impacting significantly on the resource burden and reduced overall liver disease severity (as measured by the Child Pugh and Model for End Stage Liver Disease scores) raising the possibility that its therapeutic effect may extend beyond modulating gut microbiota (Patel et al. 2014). Rifaximin, unlike vancomycin, has a lower risk of inducing bacterial resistance and is likely to be better tolerated than lactulose where non-compliance with the therapy due to the characteristic unfavourable gastrointestinal symptoms is well reported. The combination of lactulose with rifaximin has also been shown to be superior to lactulose alone in the treatment of acute HE (Courson et al. 2015).

Studies have shown probiotics to be non-inferior to lactulose in secondary prevention (Agrawal et al. 2012) and treatment of HE (Jiang et al. 2015) by preventing bacterial overgrowth and translocation and by decreasing the pH of the gut. Although no proven statistical benefit over lactulose, probiotics had a better tolerated side-effect profile (Bajaj et al. 2014a). Meta-analyses of the use of probiotics with the aim of favorably altering enteric flora towards reducing bacterial ammonia production have however shown conflicting results; one indicated an improvement in covert HE and secondary prevention of overt HE (Xu et al. 2014), however another has shown no statistically significant benefit on clinical

outcomes (McGee et al. 2011). There is agreement that there has so far been no significant negative impact with probiotic use but further wide-scale randomised controlled trials are needed to evaluate their future use.

Having identified systemic inflammation as a co-factor in HE, therapies which target the reduction in cytokine and ammonia generation potentially work to reduce the development of inflammation within the brain. It is common for patients with cirrhosis to have coexistent zinc deficiency. Zinc is a co-factor for both GS and acetyl transcarboxylase; therefore deficiency can lead to increased circulating ammonia levels through decreased metabolism (Marchesini et al. 1996; Yoshida et al. 2001). Oral zinc supplementation alone had significant improvement on the incidence and severity of HE but clinical trials have yet to be performed (Takuma et al. 2010). Conversely, excess manganese deposition in the basal ganglia and globus pallidum has been proposed to induce or enhance encephalopathic symptoms in CLD (Maffeo et al. 2014; Pomier-Layrargues et al. 1995). Molecular Adsorbents Recirculating System (MARS) may improve HE via reduction and removal of these circulating toxins thus suggesting zinc may have an additional role to play in HE.

In ALF, and HE associated with cirrhosis, albumin dialysis and plasmapheresis have been shown to have statistically significant benefit in both reduction of HE grade and resolution of symptoms (Banares et al. 2013; Stenbog et al. 2013). Studies have also shown increased speed of recovery when compared with standard medical therapy (Hassanein et al. 2007). It is thought that the removal of albumin-bound toxins has a direct effect on the neural-toxicity and inflammation via an antioxidant effect (Quinlan et al. 2005). Plasmapheresis works in a similar manner and review of the data has indicated significant improvement in overt HE and survival in ALF avoiding the need for transplantation (Stenbog et al. 2013).

In cirrhosis, the plasma amino acid profile is altered with a reduction in branch chain amino acids (BCAAs) such as leucine, isoleucine and valine (Kawaguchi et al. 2011; Marchesini et al. 2003; Muto et al. 2005). Studies looking at supplemental BCAA in patients with minimal and episodic HE have shown supplemental BCAAs have utility (Plauth et al. 1993) but it has not yet been proven to have benefit in reducing the recurrence of overt HE. A meta-analysis of more recent trials has given some support to the use of BCAAs in reducing all manifestations of HE with the supposition that BCAAs boost ammonia uptake and metabolism within skeletal muscle. BCAAs have also been proposed as an aide in the transport of ammonia nitrogen directly out of neurons (Gluud et al. 2013, 2015). There is no role whatsoever in protein restriction in the management of acute or chronic HE. Protein restriction propagates muscle catabolism and discourages muscle ammonia incorporation into glutamine (Cordoba et al. 2004).

L-Ornithine-L-Aspartate (LOLA) offers substrates for the urea cycle thus increasing glutamine synthesis and reducing ammonia levels (Acharya et al. 2009; Rose et al. 1998). In chronic HE, studies have shown superiority of LOLA when compared with lactulose in preventing episodic HE (Poo et al. 2006), inducing remission of overt HE (Kircheis et al. 2002), although has not been shown to have significant effect on minimal HE. More recent studies have examined the use of L-ornithine phenylacetate in HE in the setting of both acute and chronic liver failure which reduces cerebral edema and ammonia levels (Jalan et al. 2007; Ventura-Cots et al. 2013; Ytrebo et al. 2009). Similarly glycerol phenylbutyrate (GPB) allows increased urinary ammonia excretion in the form of phenylacetate glutamine. A study published in 2014 examining the oral administration of 6 mL GPB versus placebo for 16 weeks in patients with previous episodic HE showed significant reduction in occurrence of any HE event, as well as time to the event following treatment initiation. The significance was stronger if the patient was not already on rifaximin although this may have indicated poorer baseline (Rockey et al. 2014).

Volume expansion may have utility in reducing angiotensin II levels and ultimately increasing renal ammonia excretion in TIPSS and non-TIPSS patients. This study looked at only limited infusion of isotonic saline in stable individuals with cirrhosis and hypothesised multiple mechanisms for the effects seen including reduced renal ammoniagenesis, reduced transport of ammonia into the renal vein and increased uptake by target organs for example skeletal muscle, liver and brain (Jalan and Kapoor 2003). More recently, a double-blind controlled-trial looking at volume expansion with human albumin solution (HAS) and saline administration in patients with episodic HE showed significant improvement in survival at 90-days in patients who were administered HAS as compared with saline although no statistical improvement was seen with reduction rates of HE (Simon-Talero et al. 2013). Proposed mechanisms were reduced circulatory dysfunction and reduced oxidative stress.

Therapeutic strategies on the horizon

There appear to be tens of different options for the treatment of HE and yet day to day treatment still uses regularly only a few of these. Some are more practical, some are better understood and many of what we are discussing have only emerged in very recent years as research into this field has ballooned. So the question lies what else is on the horizon that may be utilised in practical treatment?

Cystic fibrosis, metabolic syndrome and hemochromatosis are only a few of the disease processes that can result in diabetes mellitus (DM) and liver disease together. The co-existence of diabetes causes a permanent inflammatory state via cytokine release including those already mentioned; TNF-α and IL-6 (Basu et al. 2011), and type 2 DM has been associated with HE in hepatitis C-related cirrhosis (Ampuero

et al. 2012). A study has specifically looked at the effects of metformin use in reduction of HE. The proposed mechanism was primarily better diabetic control via decreased gluconeogenesis from activation of the AMP-K pathway and modulation of TNF-α expression. The study showed partial inhibition of glutaminase activity and secondly a significant reduction in HE rates in the metformin arm versus the control arm (Ampuero et al. 2012). The drawback to metformin however is its relative contraindication and concern with the development of lactic acidosis in those with advanced cirrhosis.

Fecal microbiota transplantation (FMT) to modify enteric dysbiosis has showed significant benefit for the treatment of resistant colitis, *Clostridium difficile* infection and inflammatory bowel disease (de Vos 2013). Therefore this begs the question as to whether FMT could have a role in the treatment of HE where enteric dysbiosis has been implicated as playing an important role in its pathogenesis (Bajaj et al. 2012b, 2014b).

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